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Major Limitations in Using Element Concentrations in Hair as Biomarkers of Exposure to Toxic and Essential Trace Elements in Children

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Background: Hair is a commonly used exposure biomarker for metals and other trace elements, but concern has been raised regarding its appropriateness for assessing the internal dose.

Objectives: The aim of the present study was to evaluate children's hair as biomarker of internal dose for toxic (As, Mn, Cd, Pb) and essential elements (Mg, Ca, Fe, Co, Cu, Zn, Se, Mo).

Methods: In 207 children (9–10 years of age), originating from a population-based cohort in rural Bangladesh, we measured concentrations of the selected elements in hair (2 cm closest to the scalp) using ICP-MS. We compared these with previously measured concentrations in erythrocytes, urine, and water. For a subset of children ($n = 19$), we analyzed four consecutive 2 cm pieces of hair.

Results: There were strong associations between hair As and the other biomarkers (erythrocytes: $r_S = 0.73$, $p < 0.001$; urine: $r_S = 0.66$, $p < 0.001$); and water ($r_S = 0.60$, $p < 0.001$); and there were significant correlations between Se in hair and erythrocytes (overall $r_S = 0.38$, $p < 0.001$), and urine ($r_S = 0.29$, $p < 0.001$). Hair Co and Mo showed weak correlations with concentrations in erythrocytes. Hair Mn was not associated with Mn in erythrocytes, urine, or water, and the geometric mean concentration increased almost five times from the 2 cm closest to the head to the 7th–8th cm ($p < 0.001$). Also Mg, Ca, Co, Cd, and Pb increased from the scalp outward (>50% higher in 7th–8th cm compared with 1st–2nd cm, $p < 0.001$).

Conclusions: Hair was found to be a useful exposure biomarker of absorbed As and Se only. Of all measured elements, hair Mn seemed the least reflective of internal dose. https://doi.org/10.1289/EHP1239

Introduction

Concentrations of environmental contaminants in human hair have been extensively used to assess the individual exposure (Barbosa et al. 2013; Gibson 1980; Rodrigues et al. 2008; Schuhmacher et al. 1991; Wilhelm et al. 1994). In particular, several trace elements interact with sulfhydryl groups in the hair keratin (Hinners et al. 1974), and may therefore be found in hair at higher concentrations than in other biomarker media such as plasma, whole blood, and urine (Olmedo et al. 2010). Hair sampling offers several advantages in human biomonitoring, particularly for children because the sampling is noninvasive, does not require medical personnel or a medical clinic, and the hair samples can be stored and transported at room temperature. Also, the concentrations in hair reflect fairly long-term exposure and may pinpoint the actual time of an acute exposure, which has been repeatedly demonstrated, for example, for arsenic (As) (Koons and Peters 1994; Smith 1964; Stenehjem et al. 2007; Toribara et al. 1982). Hair may also be a useful biomarker of certain elements that occur in different chemical forms in the environment. For example, inorganic As, the most toxic form, but not the much less toxic organic As compounds prevalent in seafood, is incorporated into hair (Raab and Feldmann 2005). Recently, manganese (Mn) in hair has become an increasingly popular biomarker of exposure (Eastman et al. 2013; Gil et al. 2011; Haynes et al. 2015; Kordas et al. 2010; Lucchini et al. 2012; Wright et al. 2006), despite that it seldom correlates with Mn concentrations in blood, the most commonly used biomarker of absorbed dose or status (Gil et al. 2011; Mora et al. 2015; Rodrigues et al. 2008). However, the situation in children has not been thoroughly assessed.

Besides the incorporation in hair through the bloodstream (internal dose), metals and other trace elements may be present in hair due to external contamination from air, dust, water, or hygiene products and cosmetics (Frisch and Schwartz 2002). Washing procedures for collected hair samples prior to analysis have shown varying results (Eastman et al. 2013), and excessive washing may even remove elements from the interior of the hair (Kempson and Skinner 2012). As a consequence, the value of various trace elements in hair as biomarkers of the actual absorbed dose has been questioned (Rodrigues et al. 2008; Wołowiec et al. 2013). Still, few validation studies have been performed. The aim of the present study was to evaluate the suitability of several different trace element concentrations in children's hair for assessment of exposure to, or internal dose of, multiple toxic [As, Mn, cadmium (Cd), lead (Pb)] and essential elements, including magnesium (Mg), calcium (Ca), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo).

Materials and Methods

Study Design

This study was based on our prospective mother–child cohort with the overarching aim to elucidate effects of arsenic and other environmental pollutants on pregnancy outcomes and child health and development. The cohort was nested in a population-based randomized food and micronutrient supplementation trial in pregnancy (MINIMat; $n = 4{,}436$), carried out between November 2001 and October 2003 in a rural area southeast of Dhaka, Bangladesh (Persson et al. 2012), at which time it was realized that elevated arsenic concentrations in the wells were prevalent. At the follow-up of the children at 10 years of age (Rahman et al.

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2016), we collected hair samples for measurement of concentrations of multiple toxic and essential elements (Mg, Ca, Mn, Fe, Co, Cu, Zn, As, Se, Mo, Cd, and Pb). For evaluation of their use as exposure biomarkers (internal dose), we compared these with previously measured concentrations of the elements in blood (erythrocyte fraction) and urine (Ahmed et al. 2014), collected on average 5.0 ± 3.2 months earlier (median 6 months) (Mannan et al. 2016). In total, 207 children had complete data for all biospecimens. For 19 girls with long hair, we analyzed four consecutive pieces of the hair to compare element concentrations by distance from the scalp, and 17 of them had their blood and urine previously analyzed. We also measured elements frequently occurring at elevated concentrations in drinking water (Mg, Ca, Mn, As, and Fe) in the well water at the children's homes. The water samples were collected at the same time as the hair samples for evaluation of water used for washing as a source of contamination. Children included in the present study ($n = 207$) had slightly higher arsenic exposure compared with those not included (urine: median 74 vs. 48 μg/L; erythrocytes: 4.1 vs. 3.0 μg/kg; $p < 0.001$), but did not differ markedly in any other characteristic.

The study was approved by the research review and ethical review committees at the International Center for Diarrheal Disease Research, Bangladesh (icddr,b), and the Regional Ethical Review Board, Stockholm, Sweden, and it was performed in accordance with the Helsinki Declaration. Informed consent was obtained from all guardians, and participants were free to refrain partially or totally from the study at any time.

Sample Collection

Collection of hair samples was carried out by trained community health workers at the 10-year follow-up, which took place at the four local health clinics in Matlab following detailed written instructions. The hair specimens were cut by 18/8 stainless steel scissors from the occipital part of the child's head, beneath some covering hair, as close to the scalp as possible. The collected bundle of hair, tied at the end closest to the scalp by a nylon thread, was about the size of a match or a toothpick, thicker in case of very short hair. The hair samples were put in high-quality paper envelopes to avoid static electricity from plastic containers. For the 19 girls with fairly long hair, we measured four consecutive 2 cm pieces of each hair bundle.

Samples of water and urine were collected in plastic urine collection cups and were thereafter immediately transferred to 24 mL polyethylene bottles, which were kept cold until they, at the end of the day, were sent to the hospital laboratory and frozen at −80°C. Venous blood samples were collected from the children in sodium-heparin tubes (Vacuette, Greiner Bio-One International AG, Kremsmünster, Austria). The samples were kept cold and transported to the Matlab hospital laboratory, where plasma and erythrocytes were separated by centrifugation and stored at −20°C. All the sampling equipment was tested and found to be free of trace elements. At the end of the sampling periods, all the samples were transported in cooling boxes on ice (hair samples at room temperature) to the Karolinska Institutet, Stockholm, Sweden, for analysis of toxic and essential elements.

Trace Elements in Hair and Other Biospecimens

Samples of 2 cm long hair (always tied into a bundle with nylon thread) were washed in 50 mL 2% Triton X-100 solution in a 100 mL acid-washed glass beaker (JenaerGlas, Rasotherm, Germany) for 1 hr, rinsed 10 times with deionized water, and dried for 24 hr on acid-washed, lightweight, plastic balance boats at room temperature. Some of the boys had hair shorter than 2 cm, and for these, we used the whole sample collected. Samples (approximately 50 mg) were weighed on a calibrated (internal and external) analytical balance (Precisa 202A, Dietikon, Switzerland) with an alpha source (Staticmaster model 2U500, NRD, US) to eliminate static electric charge, which may cause inaccurate weight. The hair samples were digested in quartz tubes with 2 mL of concentrated nitric acid (65% w/w, Scharlau Trace Analysis Grade, Scharlab S.L., Sentmenat, Spain) and 3 mL deionized water for 30 min (250°C and at a pressure of 160 bar) in a Milestone ultraCLAVE II microwave digestion system (EMLS, Leutkirch, Germany). The digested solutions were allowed to cool to a temperature below 30°C, after which they were diluted with deionized water to an acid concentration of 20%. Measurement of trace elements was performed using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700x, Agilent Technologies, Tokyo, Japan), equipped with octopole reaction system collision/reaction cell technology (with He or H_2 gas) to minimize spectral interferences (Kippler et al. 2009; Lu et al. 2015). Reagent blanks were treated in the same way as the hair samples and incorporated into each analytical run. Limit of detection (LOD) was defined as three times the standard deviation of the blank values (see Table S1). Quality control was performed by inclusion of reference materials for hair (NCSZC81002b, China National Analysis Center for Iron and Steel; see Table S1).

The trace element concentrations in drinking water, urine, and erythrocytes of the children were also measured using ICP-MS. The water and urine samples were diluted 1:10 with 1% nitric acid (65% w/w, Scharlau Trace Analysis Grade, Scharlab S.L., Sentmenat, Spain). Quality control was performed by inclusion of reference material for urine (Seronorm™ Trace Elements Urine LOT 1,011,645 and 1,011,644; see Table S2). The total arsenic concentrations in urine was compared with the sum of inorganic arsenic and its metabolites methylarsonic acid and dimethylarsinic acid, measured using high-performance liquid chromatography on-line coupling with hydride generation ICP-MS. This showed strong correlation ($r_S = 0.98$; $n = 207$) (Skröder Löveborn et al. 2016), indicating negligible exposure to organic arsenic compounds through seafood. To compensate for the variation in dilution of the spot urine samples, we adjusted for mean specific gravity of the urine (1.012), measured using a digital refractometer (RD 712 Clinical Refractometer, EUROMEX microscopes, Holland) (Nermell et al. 2008).

The erythrocytes were diluted 1:25 with an alkali solution consisting of 1-butanol 2% (w/v; anhydrous, 99.8%, Sigma-Aldrich, Schnelldorf, Germany), EDTA 0.05% (w/v; H_4-EDTA, 99.995%, Sigma-Aldrich), Triton X-100 0.05% (w/v; BioXtra, Sigma-Aldrich), and sodium hydroxide 1% (NH_4OH 1%) (w/v; Romil) (Lu et al. 2015). Quality control was performed by inclusion of reference material for blood (Seronorm™ Trace Elements Whole Blood Lot 1,103,128 and 1,103,129; see Table S3). As selenium in the erythrocytes is mainly bound to hemoglobin (Hb), measured concentrations are often expressed per gram Hb (Skröder et al. 2015; Stefanowicz et al. 2013), but because the adjusted and unadjusted concentrations were highly correlated ($r_S = 0.82$, $p < 0.001$; $n = 206$), we used the unadjusted concentrations for simplicity.

Covariates

Information on maternal characteristics was obtained as previously described (Persson et al. 2012). Socioeconomic status (SES) was estimated *via* an asset index, generated through principal component analysis of household assets (on the basis of household ownership of different items) including lands, dwelling

Table 1. Element concentrations in hair (2 cm closest to the scalp), erythrocytes, urine, water [median (5th–95th percentile)], and the ratio hair/erythrocyte concentrations at 9–10 years of age ($n = 207$).

Element	Hair (mg/kg)	Erythrocytes (mg/kg)	Hair/erythrocytes	Urine (mg/L)[a]	Water (mg/L)
Magnesium	64 (31–153)	49 (41–59)	1.3 (0.62–3.1)	39 (18–74)	24 (9.4–54)
Calcium	521 (297–1,001)	11 (8.8–14)	50 (26–93)	15 (24–78)	44 (16–88)
Manganese	5.0 (1.4–23)	0.024 (0.016–0.038)	218 (58–883)	0.18 (0.039–3.1)b	0.41 (0.27–3.2)
Iron	72 (34–186)	816 (692–862)	0.087 (0.043–0.24)	0.0044 (0.0019–0.019)	2.4 (0.089–8.0)
Cobalt (µg/kg or µg/L)	30 (12–90)	0.086 (0.050–0.15)	319 (141–1043)	0.27 (0.13–0.72)	–
Copper	8.9 (6.7–12)	0.66 (0.56–0.77)	14 (10–18)	0.0074 (0.0036–0.015)	–
Zinc	154 (109–200)	9.6 (7.1–12)	16 (11–24)	0.21 (0.073–0.54)	–
Arsenic	0.53 (0.14–2.9)	0.0041 (0.0015–0.22)	118 (45–308)	0.074 (0.022–0.42)	5.6 (0.11–381)[b]
Selenium	0.53 (0.37–0.66)	0.18 (0.14–0.22)	2.9 (2.0–3.8)	0.014 (0.0076–0.027)	–
Molybdenum (µg/kg or L)	41 (27–83)	0.57 (0.25–1.5)	73 (25–223)	50 (26–105)	–
Cadmium (µg/kg or L)	29 (0.76–150)	0.91 (0.45–2.1)	31 (7.5–271)	0.30 (0.11–0.79)	–
Lead	1.6 (0.50–6.4)	0.11 (0.071–0.21)	15 (3.8–48)	0.0014 (0.00067–0.0029)	–

[a]Adjusted to specific gravity (1.012).
[b](µg/L).

characteristics, and household sanitation (Gwatkin DR et al. 2000). The SES data of the families was updated at the follow-up of the children at 9–10 years of age. Children's anthropometry was measured at birth according to standard procedures (Persson et al. 2012). The body weight of the children at 9–10 years of age was measured to the nearest 0.1 kg with a digital scale (TANITA HD-318, Tanita Corporation, Japan), and height was measured with a locally manufactured wooden stadiometer (precise to 0.1 cm). The measured weight and height were converted to weight-for-age z-scores (WAZ), BMI-for-age z-score (BAZ), and height-for-age z-scores (HAZ), using the WHO growth reference for school-aged children and adolescents (de Onis et al. 2007). Underweight was defined as $\text{WAZ} < -2$ (below -2 standard deviations from median weight-for-age of reference population) and stunting was defined as $\text{HAZ} < -2$ (below -2 standard deviations from median height-for-age of reference population). Season of biospecimen sampling was defined as pre-monsoon (January–May), monsoon (June–September), or post-monsoon (October–December). Children's Hb concentrations (g/L) were measured in a drop of venous blood using a HemoCue photometer (HemoCue AB, Ängelholm, Sweden).

Statistical Analyses

Statistical analyses were performed using the software Stata/IC (version 12.1; StataCorp). Data distribution was evaluated using histograms, scatter plots with smoothed lowess lines, and box plots. To assess potential variations in the concentration of elements in hair depending on the distance from the scalp (along the length of the hair), we used Friedman's test.

Associations between the element concentrations in hair and those in erythrocytes, urine, and water were initially evaluated using scatter plots, Spearman's rank correlation coefficients (r_S), and principal component analyses. Because urine and blood were often collected before hair and water, we further assessed whether correlations between the biomarkers differed depending on the time between the sampling periods by stratifying the correlation analyses by the time difference (median split at 6 months; two hair samples were missing a collection date). For the children with four consecutive pieces of hair analyzed (up to ~ 8 cm from the head; $n = 19$), we assessed the correlations for each 2 cm of hair ($n = 17$ with erythrocytes and urine analyzed). For As, we also assessed whether the correlations between hair and erythrocyte concentrations varied depending on the source of As by stratifying on water As <10 µg/L (exposure mainly from food) or >50 µg/L [exposure mainly from water (Kippler et al. 2016)].

Elements with significant correlations ($p < 0.05$) between the concentrations in hair and erythrocytes were further assessed with linear regression analyses to evaluate potential influencing factors. The models included covariates that correlated ($r > 0.10$ and $p < 0.10$) with any of the included element concentrations in hair or erythrocytes, that is, child age, gender, Hb, number of siblings, season of blood sampling, and family SES. We also included the difference between sampling time points (months, continuous) to see whether this had an impact on the associations. The distribution of all elements in hair, erythrocytes, urine, and water were skewed (except for Se in hair and erythrocytes). Therefore, we compared the results with $\log_2$-transformed concentrations in the linear regression models.

Results

The children were on average 9.5 years old at hair collection, had on average 1.2 siblings, and 49% were girls. Of all children, 26% were stunted ($\text{HAZ} < -2$) and 42% were underweight ($\text{WAZ} < -2$). Table 1 shows concentrations of all analyzed elements in hair, erythrocytes, urine, and water ($n = 207$), as well as the ratio between hair and erythrocyte concentrations. The largest differences in concentration between hair and erythrocytes were found for Co (median 319 times higher concentration in hair than in erythrocytes), Mn (218 times higher in hair) and As (118 times higher in hair). The only element with a higher concentration in erythrocytes than in hair was Fe (median ratio hair/erythrocytes = 0.09). All hair samples were black and none seemed to be dyed based on visual examination of the paper envelopes (often discolored in the case of dyed hair) and the acidified samples (color released in the case of dyed hair) prepared for digestion. Compared to girls, boys had lower concentrations of Mg (geometric mean 56 vs. 75 mg/kg in boys and girls, respectively), Ca (482 vs. 595 mg/kg), and As (0.52 vs. 0.61 mg/kg) in the hair. HAZ, WAZ, or BAZ did not correlate with any of the element concentrations in hair.

When comparing concentrations by distance from the scalp, we found that the concentrations of Mg, Ca, Mn, Co, Cd, and Pb were more than 50% higher in the fourth sample (7–8 cm from the head) compared with the first (1–2 cm from the head; Table 2; Figure 1; see also Figure S1). The largest increase was for Mn, for which the geometric mean concentration was 4.6 times higher in the fourth than in the first sample (closest to the head). One child (girl) had much higher Mn concentrations in all the hair subsamples compared to the other children (Figure 1) and that child also had a very high concentration of Mn in the drinking water (4.5 mg/L) but a normal erythrocyte concentration (0.029 mg/kg). The same child also had very high Co concentrations in all hair subsamples (Figure 1), although the concentrations of Co in urine (0.27 µg/L) and erythrocytes (0.073 µg/kg) were similar to the other children (median 0.27 µg/L and

Table 2. Geometric mean (range) concentrations (mg/kg) of elements in hair by hair length (2 cm pieces from head outward) at 9–10 years of age ($n = 19$).

Element	1st–2nd cm	3rd–4th cm	5th–6th cm	7th–8th cm	7th–8th/1st–2nd cm	p-value[a]
Magnesium	85 (31–234)	129 (49–455)	199 (68–429)	221 (74–434)	2.6	<0.001
Calcium	610 (311–1,418)	796 (41–1,873)	1,101 (619–2,151)	1,283 (613–2,184)	2.1	<0.001
Manganese	6.9 (1.2–51)	16 (5.9–96)	27 (10–162)	32 (11–209)	4.6	<0.001
Iron	85 (40–285)	106 (50–230)	113 (59–220)	121 (70–209)	1.4	<0.001
Cobalt	0.040 (0.018–0.19)	0.062 (0.023–0.46)	0.079 (0.030–0.64)	0.10 (0.033–0.88)	2.5	<0.001
Copper	9.7 (6.5–17)	11 (7.1–15)	11 (7.7–21)	12 (7.8–30)	1.2	<0.001
Zinc	151 (99–195)	171 (114–262)	199 (126–380)	215 (116–674)	1.4	<0.001
Arsenic	0.67 (0.12–3.1)	0.51 (0.087–2.4)	0.45 (0.093–1.6)	0.38 (0.092–1.6)	0.57	<0.001
Selenium	0.53 (0.41–0.68)	0.52 (0.28–0.76)	0.51 (0.37–0.71)	0.46 (0.34–0.68)	0.87	<0.001
Molybdenum	0.044 (0.028–0.067)	0.050 (0.033–0.082)	0.054 (0.036–0.081)	0.054 (0.033–0.079)	1.2	<0.001
Cadmium	0.031 (0.012–0.56)	0.054 (0.027–0.59)	0.070 (0.029–0.69)	0.087 (0.041–0.49)	2.8	<0.001
Lead	1.7 (0.87–4.3)	2.4 (0.89–15)	2.6 (0.68–13)	3.2 (1.1–15)	1.9	<0.001

[a]Friedman's test.

0.086 μg/kg in urine and erythrocytes, respectively). She was also one of the two children with very high hair Pb concentrations (Figure 1), and again, concentrations in erythrocytes and urine were comparable to the other children. In contrast, the child (also a girl) with high hair Cd concentrations in all samples also had high Cd concentrations in urine (0.79 μg/L) and erythrocytes (1.9 μg/kg). The only elements with slightly decreasing concentrations by hair length were Se and As, the concentrations of which were 13% and 43% lower in the fourth sample than in the first sample, respectively (Table 2; see also Figure S1).

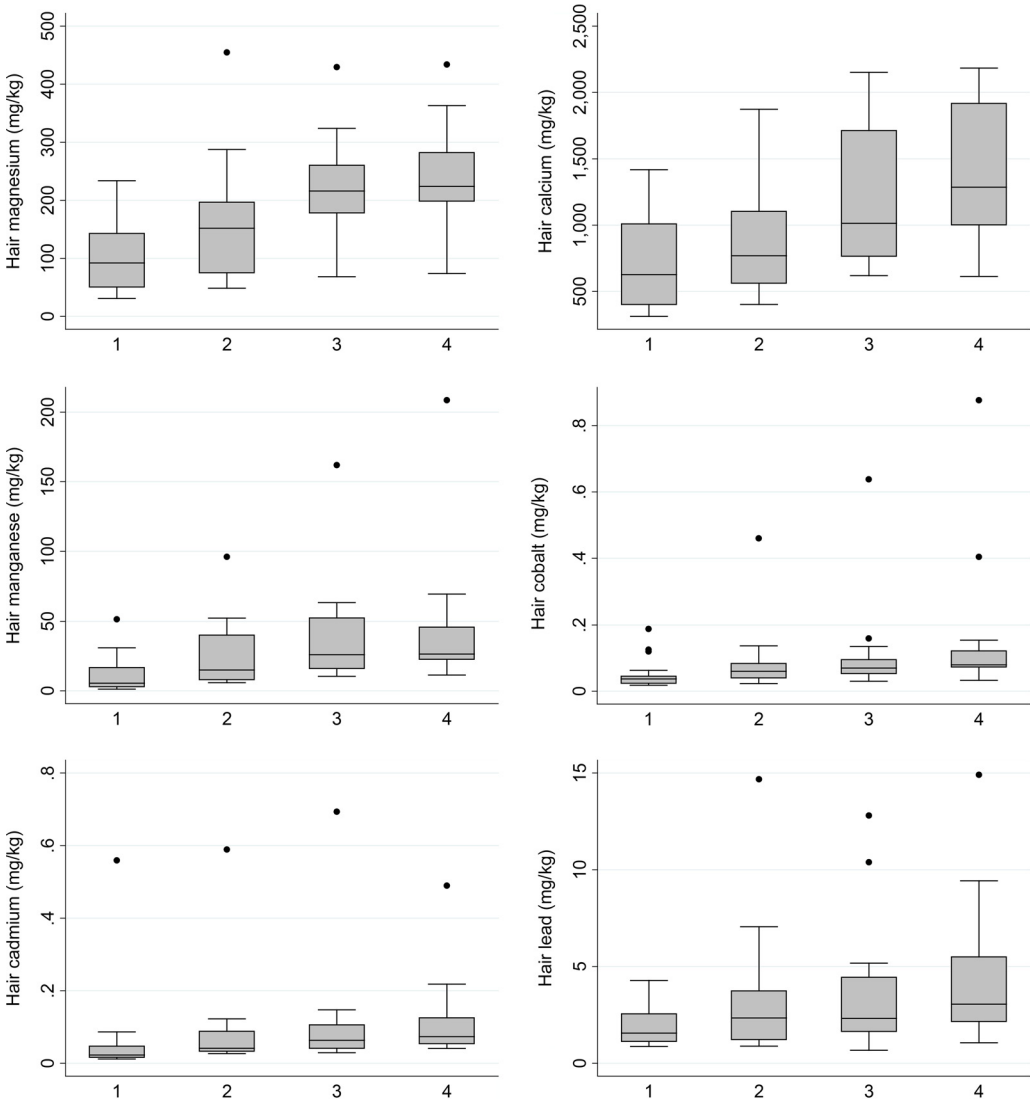

Figure 1. Box plots of concentrations of various elements in children's hair (mg/kg), by distance from the head (one step equals 2 cm; $n = 19$). The boxes represent the first and third quartiles (inner quartile range; IQR), and the band inside represents the median. The whiskers represent the lowest and highest value within 1.5 times the IQR. Dots represent outliers.

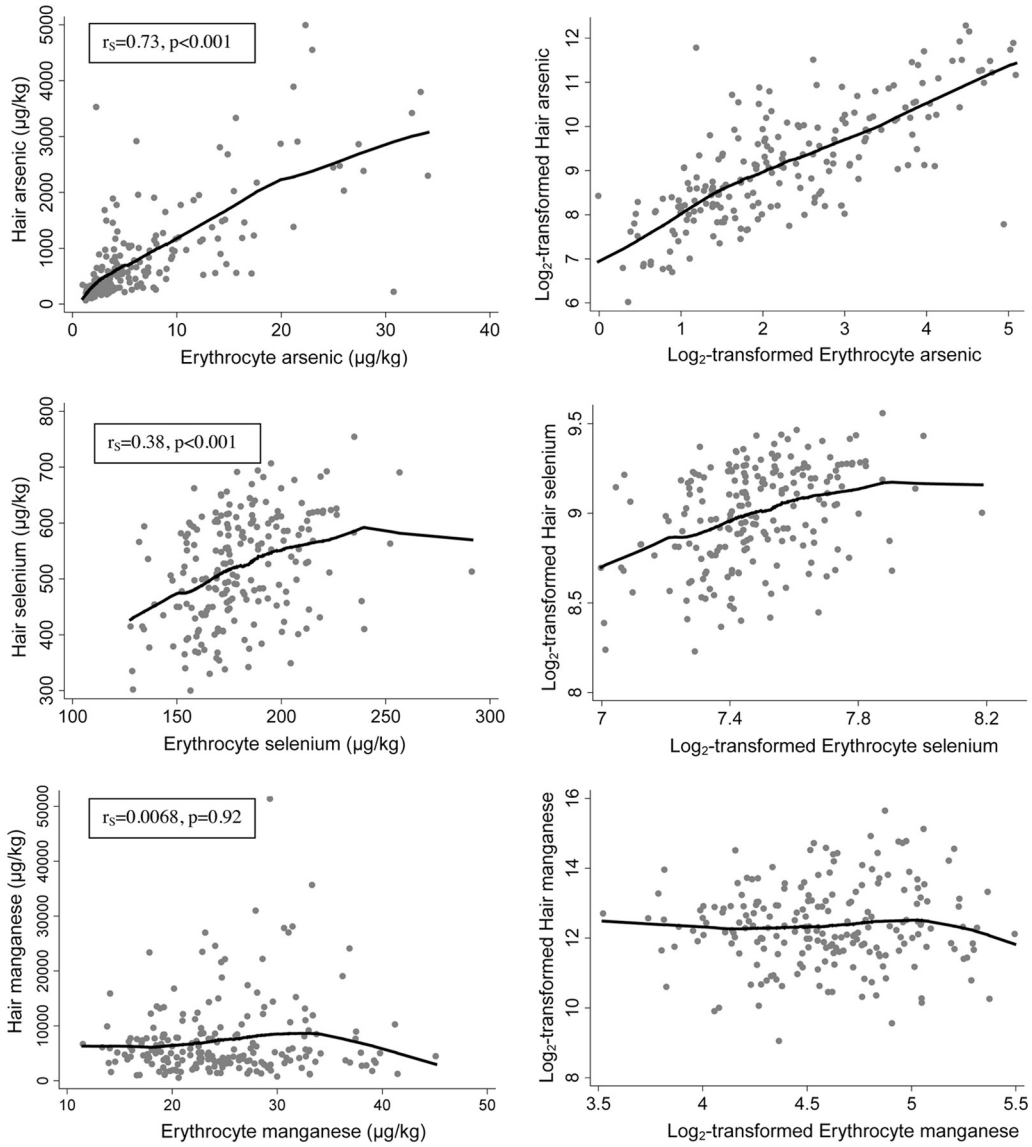

Figure 2. Scatter plots with smoothed lowess lines and Spearman correlation coefficients (r_S, p-value) for children's concentrations of arsenic (top), selenium (middle), and manganese (bottom) in hair and erythrocytes at 9–10 years of age.

We found strong correlations between concentrations of As in hair with those in erythrocytes ($r_S = 0.73$, $p < 0.001$; Figure 2; Table 3), urine ($r_S = 0.66$, $p < 0.001$), and water ($r_S = 0.60$, $p < 0.001$), and all associations appeared more or less linear (see Figure S2). When stratifying these analyses on the time between collection of hair and erythrocytes/urine (median split at 6 months; $n = 107 \leq 6$ months, $n = 98 > 6$ months), we found only slightly stronger correlation for the children with shorter time between the collection time points (Table 3). Comparison of children with water As <10 and >50 µg/L showed similar correlations between hair and erythrocyte As in both groups ($r_S = 0.59$ and 0.58, respectively). The multivariable-adjusted linear regression analyses, both with nontransformed and $\log_2$-transformed concentrations, also showed a strong association between the concentrations of As in hair (first 2 cm) and erythrocytes ($p < 0.001$; Table 4). An increase in erythrocyte As of 1 µg/kg (corresponding to 20 µg/L in urine) was associated with 93 µg/kg higher As concentration in the children's hair. None of the covariates seemed to have an impact on the association between hair and erythrocyte concentrations (difference between crude and adjusted estimate <10%).

Similarly, hair Se correlated significantly with erythrocyte Se ($r_S = 0.38$, $p < 0.001$; Figure 2 and Table 3), as well as urinary Se ($r_S = 0.29$, $p < 0.001$; Table 3). The correlation with erythrocyte Se was similar for children with shorter time between the sampling points (Table 3). When restricting the correlation analyses to the children with four consecutive hair segments analyzed (increasing distance from the scalp), we found that the association between erythrocyte Se and hair Se was stronger for the 7th–8th cm of hair (corresponding to incorporation from blood around the same time as the blood collection; $r_S = 0.54$, $p = 0.026$), and also for the 5th–6th cm of hair ($r_S = 0.39$, $p = 0.099$), compared with the first 2 cm (corresponding to intake about 6–7 months after blood collection; $r_S = 0.27$, $p = 0.26$; $n = 17$). The multivariable-adjusted linear regression analyses also showed positive associations between hair and erythrocyte Se ($p < 0.001$; Table 4), and again, none of the covariates had a large impact on the association (difference between crude and adjusted estimate <10%). An increase in erythrocyte Se of

Table 3. Spearman correlations (p-value) between elements in hair, erythrocytes, urine, and water at 9–10 years of age ($n = 205$) stratified by time difference between collection of erythrocytes/urine, and hair/water.

	Erythrocytes		Urine		Water
Element	≤6 Months	>6 Months	≤6 Months	>6 Months	All
Magnesium	0.032 (0.76)	−0.049 (0.63)	−0.0064 (0.95)	−0.036 (0.72)	0.18 (0.0090)
Calcium	0.078 (0.42)	0.16 (0.12)	−0.11 (0.26)	0.0031 (0.98)	0.032 (0.65)
Manganese	−0.082 (0.40)	0.088 (0.39)	0.017 (0.87)	0.19 (0.061)	0.078 (0.26)
Iron	0.038 (0.70)	−0.13 (0.19)	0.14 (0.14)	0.0057 (0.96)	0.14 (0.045)
Cobalt	0.42 (<0.001)	0.070 (0.49)	0.22 (0.025)	0.13 (0.20)	NA
Copper	−0.11 (0.26)	0.19 (0.055)	−0.0037 (0.97)	0.14 (0.17)	NA
Zinc	−0.045 (0.64)	−0.13 (0.20)	0.045 (0.64)	−0.23 (0.023)	NA
Arsenic	0.80 (<0.001)	0.66 (<0.001)	0.71 (<0.001)	0.58 (<0.001)	0.59 (<0.001)
Selenium	0.37 (<0.001)	0.44 (<0.001)	0.26 (0.0068)	0.42 (<0.001)	NA
Molybdenum	0.081 (0.038)	0.23 (0.022)	0.26 (0.0062)	0.24 (0.018)	NA
Cadmium	0.087 (0.38)	−0.0018 (0.99)	0.16 (0.11)	0.12 (0.22)	NA
Lead	0.040 (0.68)	0.15 (0.14)	0.0075 (0.94)	0.067 (0.51)	NA

Note: Median split at 6 months; $n = 107 \leq 6$ months, $n = 98 > 6$ months. NA, not applicable.

1 μg/kg was associated with an increase of 1.5 μg/kg in the hair (Table 4). Hair Se concentrations were somewhat higher during pre-monsoon season (median 552 μg/kg), compared with monsoon (median 444 μg/kg) and postmonsoon (median 492; $p < 0.001$).

There were significant, positive associations, although weak, between the different biomarkers of Co and Mo (Tables 3 and 4). Again, the covariates did not seem to have any influence on the associations for Mo, but the associations for Co were almost 50% weaker after adjustments. There was no correlation between the concentrations of Mn in hair and erythrocytes ($r_S = 0.0068$, $p = 0.92$; Figure 2), or between Mn in hair and water ($r_S = 0.067$, $p = 0.33$; Table 3). Also the remaining elements were either weekly or nonsignificantly correlated with the other biomarkers (Table 3), and were thus not evaluated further by linear regression analyses.

The strongest inter-element correlations in hair were observed between Mg, Ca, Mn, and Co ($r_S = 0.43 - 0.76$; see Figure S3, and Table S4). This was further evident in the principal components analysis (see Figure S3, bottom). Also, Cd, Pb, and Mn correlated positively, and Cd and Pb correlated inversely with Zn.

Discussion

Few studies have evaluated the suitability of hair as a biomarker of trace element exposures, especially for children. In this evaluation in rural Bangladeshi children, hair As and Se showed the strongest associations with their respective concentrations in other exposure biomarkers (erythrocytes and urine; representing the absorbed dose). We also found weak associations between Co and Mo in hair, erythrocytes and urine, whereas there were no associations for Mg, Ca, Mn, Fe, Cu, or Zn. Neither was there any association for Cd or Pb. Importantly, the concentration of all the latter elements, and also Co, increased with hair length (from the scalp outward, i.e., with increasing age of the hair), indicating contamination by dust, water used for washing, shampoos, or other hair cosmetics that was not removed by the washing procedure used for the collected hair samples (based on slightly alkaline Triton X-100 solution). Obviously, older parts of the hair (more distant from the scalp) have been exposed to water and airborne dust for much longer time than the first 1–2 cm from the scalp, enabling a higher extent of binding from external sources over time. The largest difference was for Mn, for which the geometric mean hair concentration increased by almost five times from the first 2 cm sample to the fourth, supporting that it does not reflect the internal dose.

Many of the children had elevated concentrations of As in their hair (median 0.53 mg/kg, range 0.07–5 mg/kg), which was expected because of the prevalent contamination of drinking water and rice, the main staple food (Kippler et al. 2016), and the high affinity of As for sulfhydryl groups, present in the keratin. The correlation with erythrocyte As in the children with low water As (<10 mg/L) was similar to that in children with elevated water As concentrations (>50 μg/L), indicating that hair As is an equally good indicator of the intake of inorganic arsenic through rice as that through drinking water. Similar concentrations of As in hair (average 0.93 mg/kg) have been reported for children living in a contaminated area in Cambodia (300–500 μg As/L in water) (Vibol et al. 2015). Usually, the background concentration of hair As is well below 0.2 mg/kg, while concentrations as high as 54 mg/kg has been found in severely intoxicated persons (Stenehjem et al. 2007). We found no increase in the hair As concentration with increasing distance from the scalp, indicating no remaining external contamination after the alkaline washing procedure prior to analysis and that the hair As indeed reflects the internal dose. In support, the correlation between hair and water arsenic ($r_S = 0.60$) was rather similar

Table 4. Multivariable-adjusted linear regression analyses for associations between hair (mg/kg) and erythrocyte (mg/kg) concentrations of arsenic, selenium, cobalt, and molybdenum of children at 9–10 years of age ($n = 204$).

	Crude		Adjusted[a]	
Element	β (95% CI)	p-value	β (95% CI)	p-value
Arsenic (nontransformed)	93 (81, 105)	<0.001	92 (80, 105)	<0.001
Arsenic ($\log_2$-transformed)	0.80 (0.70, 0.90)	<0.001	0.79 (0.69, 0.90)	<0.001
Selenium (nontransformed)	1.4 (0.96, 1.9)	<0.001	1.5 (1.0, 1.9)	<0.001
Selenium ($\log_2$-transformed)	0.55 (0.37, 0.72)	<0.001	0.55 (0.38, 0.72)	<0.001
Cobalt (nontransformed)	90 (−3.7, 184)	0.060	46 (−50, 142)	0.34
Cobalt ($\log_2$-transformed)	0.32 (0.10, 0.53)	0.004	0.19 (−0.021, 0.41)	0.077
Molybdenum (nontransformed)	17 (3.1, 32)	0.018	15 (0.96, 30)	0.037
Molybdenum ($\log_2$-transformed)	0.13 (0.031, 0.22)	0.010	0.10 (0.0093, 0.20)	0.031

[a]Adjustments: age, gender, SES, hemoglobin, number of siblings, season of erythrocyte sampling, and difference between sampling time points.

to that with erythrocytes ($r_S = 0.73$) and urine ($r_S = 0.66$). Importantly, there was a strong correlation between As in hair and erythrocytes even for children with the longest time difference between the sampling periods, suggesting that the As exposure does not vary much over time in this population, in accordance with our recent evaluation of urine As over time in the same children (Kippler et al. 2016).

There are numerous studies on hair Se in the literature, but few comparisons with other biomarkers (Ashton et al. 2009). The present study provides support for the use of Se concentrations in hair as a biomarker of Se status in children, as previously suggested for adults (Ashton et al. 2009; Högberg and Alexander 2007; Lemire et al. 2009). The association between the concentrations in hair and erythrocytes was stronger among children with hair and blood samples representing the same period in time [i.e., the 5th–6th and 7th–8th cm of hair and blood collected on average 5 months (median 6 months) prior to hair collection], which is expected because both hair (first 2 cm) and erythrocyte Se reflect the exposure during the previous 2–3 months. The variation in the correlation by the duration between sampling time points was probably related to a varied intake of Se across the year. The intake of Se is mainly from the diet, with little contribution from drinking water (<0.1 μg/L in the study area, M. Vahter, unpublished data, 2017). The pre-monsoon season has the highest food security in rural Bangladesh (Hillbruner and Egan 2008) and likely the highest Se intake. Indeed, the hair Se concentrations were higher during premonsoon season.

The concentrations of hair Se in the present study (0.30–0.75 mg/kg) are difficult to compare with previous studies because of major differences in methods for hair sampling, washing, and analytical method, as well as in the sample size of study groups. Still, the fact that 95% of all children had hair Se concentrations above 0.36 mg/kg, the concentration reported for adults in Se-adequate areas (Högberg and Alexander 2007), is surprising considering that almost 60% of the mothers in the present study population were Se deficient during pregnancy (Skröder et al. 2015), and 92% of the children had urinary Se concentrations below 26 μg/L, the concentration found in Se-adequate areas (Högberg and Alexander 2007). In Brazilian 4.6-year-old children with much higher plasma concentrations (range 47–172 μg/L), the average hair Se concentration was only 0.31 mg/kg (Martens et al. 2015). Furthermore, the children's hair to erythrocyte Se ratio (2.9) in the present study appears very high in comparison with the hair to whole blood ratio of 2.8 observed in men and women in the Amazon region of Brazil with very high blood Se concentrations (range 142–2,247 μg/L) (Lemire et al. 2009). Given that Se concentrations in erythrocytes are higher than in plasma (mainly due to the binding of Se to hemoglobin), the ratio of hair to whole blood Se for the present children would have been much higher than in the Brazilian individuals. In addition, a Chinese study reported an average hair Se concentration of 0.32 mg/kg among individuals ($n = 366$) with Keshan Disease (caused by selenium deficiency) and 0.45 mg/kg among 200 controls (Yang et al. 2010). In the present study, only 27% of the children had hair Se concentrations below 0.45 mg/kg. The reason for the high hair Se excretion in the malnourished children in Bangladesh needs to be elucidated. The varying hair Se concentrations also across studies with normal Se intake or plasma concentrations suggest either a large difference in Se tissue distribution depending on possible genetic variations, age, or other factors such as speed of hair growth and type of diet (form of dietary Se). Also, hair Se concentrations may differ depending on the methods for sample preparation and analysis. Indeed, a previous study found huge interlaboratory variations when measuring hair concentrations of multiple elements (Seidel et al. 2001).

In the present population, there is a continuous change to deeper wells with lower As concentrations but sometimes high Mn concentrations (Kippler et al. 2016). The poor associations between hair Mn and the other biomarkers in the present study show that the levels of Mn in hair did not reflect the actual internal dose. The marked change in Mn concentration along the hair length, and the high water concentration but normal erythrocyte concentration of the outlier in Figure 1, strongly points to external contamination. Still, we found no correlation between hair Mn and water Mn concentrations. The problem of using hair Mn as a biomarker has also been concluded in a Brazilian study on 280 adults, for which there was no correlation between hair and blood Mn (Rodrigues et al. 2008). A Canadian study found a positive correlation between very low water (median 31 μg/L) and hair Mn concentrations (Bouchard et al. 2011). However, the authors found no association between the wide range of Mn in food (median 2,335 μg/day) and concentrations in hair, suggesting either that the Mn in hair was affected by external contamination, or that there were differences in Mn metabolism, depending on source, as suggested by the authors (Bouchard et al. 2011). The intestinal uptake of Mn^{2+} is assumed to occur *via* the same transporters, whether the ions originate from water or food, although the bioavailability may differ. To note, the washing procedures of the hair samples prior to analysis have been shown to have great impact on the Mn concentration (Eastman et al. 2013; Kempson and Skinner 2012), and the ideal way of washing the hair samples is yet to be determined. Also hair Cd has been used as exposure biomarker in studies on both children and adults (Jin et al. 2016; Kordas et al. 2015), although it was concluded long ago that hair Cd does not reflect the internal dose (Wilhelm and Idel 1996), which was also found in the present study.

Strengths of the present study include the analyses of multiple toxic and essential elements being done by ICP-MS, the use of different biospecimens for evaluation of the appropriateness for the absorbed dose of each element, and analyses of multiple subsegments of the same hair, representing different ages of the hair.

The main limitations of this study include the lack of plasma samples, lack of information on personal habits regarding hair care, and also the lack of detailed data on dietary habits. In the absence of external contamination, concentrations in both hair and erythrocytes are reflective of the previous months' exposure, and thus the optimal sampling procedure would be to collect blood/urine about 2–3 months prior to hair collection. However, in the present study there were on average 5 months between urine/blood collection, and hair collection. Indeed, our data indicated stronger correlations with blood and urine for children whose hair samples were closer in time to the other specimen. The children with multiple hair samples ($n = 19$) analyzed were only girls. Many boys had very short hair (even less than 2 cm), which might explain the somewhat higher concentrations of some elements (with increasing concentration with distance from the head), in the girls' hair compared with the boys'.

Conclusion

Although the idea of measuring toxic and essential elements in children's hair is tempting, the present study shows that this is only appropriate for assessment of the internal dose of As and Se. As the concentrations of As and Se did not seem to be affected by external contamination after washing the samples using Triton X, it would be possible to use specific segments of the hair to assess the exposure or intake over time, or at a certain point in time. Manganese in the present children's hair did not seem to reflect internal dose, and should therefore not be used as a biomarker of

internal dose. If used as exposure marker in future studies, the appropriateness has to be proven.

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